thyroxine and triiodothyronine,[15] some have also reported the presence of iodotyrosine in the peripheral blood of thyrotoxic or normal humans, or those stimulated by thyrotropin.[16] The present findings that 3-iodo-L-tyrosine lowers the catecholamine levels *in vivo* raises the question whether thyroid hormones affect the biosynthesis of catecholamines *in vivo*.

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Action of angiotensin on myocardial and renal catecholamines in the rabbit

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THERE have been several recent reports indicating the necessity of an intact sympathetic nervous system for the cardiovascular action of angiotensin.[1–4] Benelli *et al.*[2] have reported the involvement of the peripheral sympathetic nerves and have hypothesized that angiotensin acts at the peripheral nerve endings by promoting a greater output of norepinephrine. There have also been suggestions that angiotensin has a specific storage pool or might exchange with or replace norepinephrine stores in tissues.[3] On the other hand, Zimmerman *et al.*[4] have reported no definite indication that the release of norepinephrine during nerve stimulation was facilitated by angiotensin.

Regarding the action of angiotensin on the heart, Youmans *et al.*[5] observed that the positive chronotropic effect of angiotensin in the ganglionically blocked dog was inhibited by bretylium. These authors conclude that this effect of angiotensin is dependent on catecholamines. In the studies of Gross and co-workers,[6] reserpine pretreatment for 3 days greatly diminished the positive inotropic effect of angiotensin in rats as evaluated by its effect on cardiac output.

Fowler and Holmes,[7] on the other hand, observed that reserpine treatment did not change the positive inotropic effect of angiotensin. Similar results have been reported by Koch-Weser[8] on the isolated cat papillary muscle. There is good evidence that angiotensin is a potent releaser of catecholamines from the adrenal medulla.[9–10]

The above considerations prompted us to study the effect of intravenous injections and infusions of angiotensin on myocardial and renal catecholamine levels in an attempt to further define the relationship between angiotensin and the sympathetic neurotransmitter norepinephrine.

METHODS

All experiments were carried out on white rabbits of either sex weighing from 1·4 to 3·0 kg. A total of 110 rabbits were used. For the injection experiments various doses of synthetic angiotensin (Hypertensin, CIBA) were injected via an ear vein and the animals sacrificed at 2, 5, 10, 30, or 60 min by a blow on the head. The heart and right kidney were immediately removed, washed with saline, weighed, and extracted with 5% trichloroacetic acid. The homogenates were then filtered by suction and the clear filtrates adsorbed on alumina according to the method of Euler and Lishajko.[11] After elution with 0·2 N acetic acid the samples were analyzed for epinephrine and norepinephrine on the autoanalyzer, according to the automated trihydroxyindole method of Watts and Robinson.[12]

Other experiments were carried out in a similar manner except that angiotensin was infused into an ear vein via a pediatric scalp vein infusion set and a Harvard infusion pump. Two different total doses were infused: 3·0 and 6·0 μg/kg for 5, 10, or 20 min. The animals were then sacrificed in a similar manner at 2, 5, or 10 min after the end of infusion. The tissues were homogenized and analyzed as described above. Values obtained after the injection or infusion of saline served as control values. Recovery was found to be between 80% and 90%. No correction has been made for the 10–20% amines lost during the procedure.

Other experiments were conducted in which the blood pressure was continuously monitored by means of a cannulated femoral artery connected to a Statham transducer and a Grass polygraph. Heart rate changes were also observed by means of a Grass tachograph. The blood pressure and heart rate were recorded for various times after the injection or infusion of angiotensin in an attempt to correlate these changes with tissue catecholamine levels.

Statistical analysis for significance of data was made by Fisher's t test.

RESULTS AND DISCUSSION

The time–response pattern of the i.v. injection of 0·3, 1·0, and 3·0 μg angiotensin/kg on heart norepinephrine levels in the rabbit are depicted in Fig. 1. It can be seen that all three doses produced an initial increase in amine levels appearing at 2 min after injection. These values were all statistically significant ($P < 0·05$). This increase was followed by a slight decrease which approached statistical significance for the two lower doses at 30 min ($P = 0·05$). All values had returned to control or slightly greater than control by 60 min.

Figure 2 shows the results of the infusion of angiotensin in a total dose of 3 μg/kg for a period of

TABLE 1. THE EFFECT OF THE INTRAVENOUS ADMINISTRATION OF ANGIOTENSIN ON MEAN ARTERIAL BLOOD PRESSURE, HEART RATE, AND MYOCARDIAL NOREPINEPHRINE LEVELS IN MINUTES AFTER THE INJECTION

Values are expressed as mm Hg, beats/min, and μg/g respectively.

Angiotensin dose (μg/kg)	Mean B.P.	H.R.	NE	Mean B.P.	H.R.		Mean B.P.	H.R.	NE
	Control			1 Min			2 Min		
0·3	104	190	1·6	138	170		117	190	2·36
1·0	76	166	1·6	131	108		74	166	2·12
3·0	70	144	1·6	135	100		72	210	2·05
	5 Min			10 Min			30 Min		
0·3	104	175	1·29	103	185	1·27	104	195	1 23
1 0	67	168	1 45	77	164	1·39	78	155	1·11
3·0	55	140	1·53	60	150	1·24	68	146	1·46

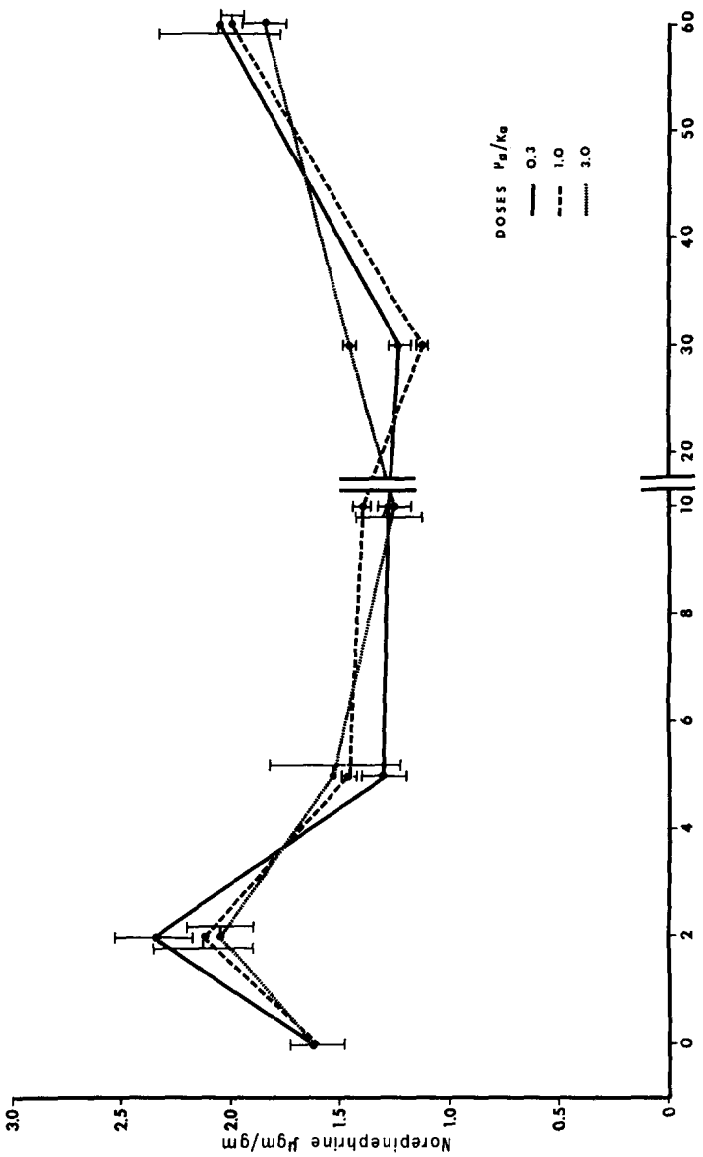

FIG. 1. The effect of the intravenous injection of angiotensin on myocardial norepinephrine levels in the rabbit. Doses in μg/kg were administered singly and tissues removed and analyzed at time in minutes listed on the abscissa. All values are recorded on the ordinate as μg/g. Each circle is the mean of at least 4 experiments.

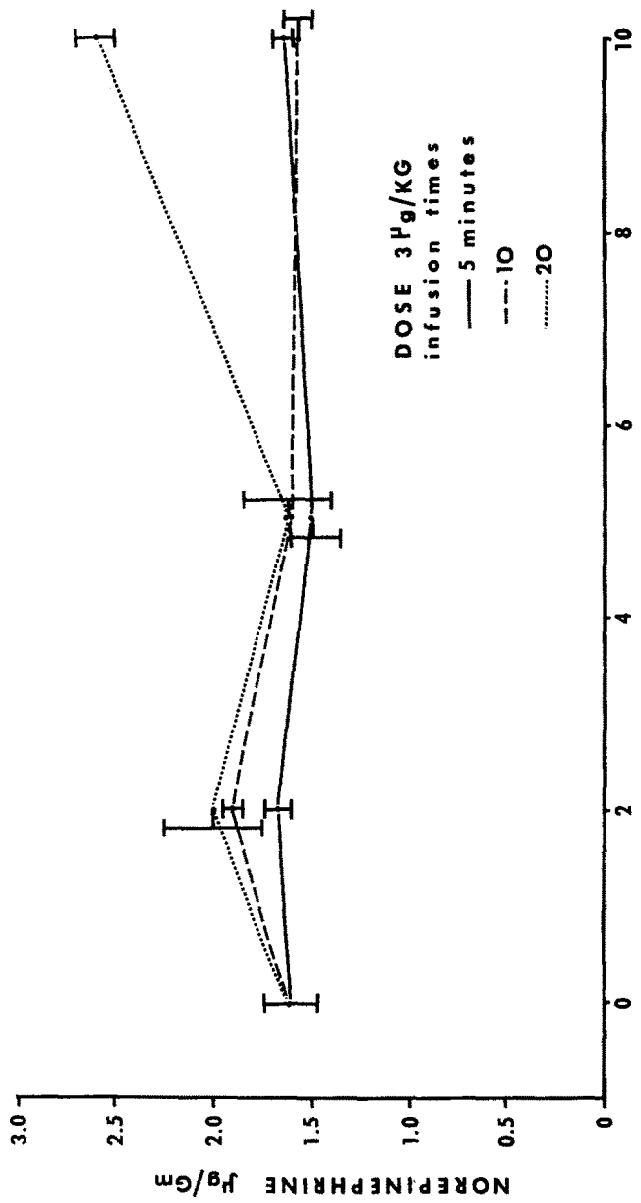

FIG. 2. The effect of the intravenous infusion of angiotensin on myocardial norepinephrine levels in the rabbit. A total dose of 3μg/kg was infused for 5, 10, or 20 min and tissues removed and analyzed at time in minutes listed on the abscissa. Norepinephrine levels are recorded on the ordinate as μg/g. Each circle is the mean of at least 4 experiments.

5, 10, or 20 min on heart norepinephrine levels. For the two shorter infusion periods a slight increase was seen, followed by a return to preinfusion values by 10 min. The longest infusion time of 20 min produced slightly elevated values which became highly significant 10 min after the end of infusion ($P < 0.001$).

Table 1 represents a summary of the effect of the injection of three doses of angiotensin on mean arterial blood pressure, heart rate, and myocardial norepinephrine levels for a period up to 30 min after administration. It can be seen that all three doses produced an immediate but transient pressor response. A similar transient reflex bradycardia was also observed. Both the pressor and heart rate changes had returned to preinjection levels by 5 min.

The results of the effect of intravenous injections or infusions of angiotensin on renal norepinephrine or epinephrine levels indicated that there was no change from control values. There was likewise no significant change in heart epinephrine levels after angiotensin treatment.

The increase in norepinephrine levels of the rabbit heart observed in these experiments could possibly be caused by an increased synthesis rate of norepinephrine due to central stimulation, as observed by Buckley *et al.*[13] and Smookler and Buckley.[14] In addition, there may be an inhibition of sympathetic tone resulting in a decrease in the spontaneous release of norepinephrine, owing to the severe vasoconstriction and reflex bradycardia produced by angiotensin. It must be pointed out, however, that such a synthesis rate would be extraordinarily high. Another possible contribution is that of amines released from the adrenal medulla and subsequently taken up into cardiac stores.

These present experiments give no definite indication that angiotensin releases norepinephrine from adrenergic nerve endings.

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